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Developing methods for emerging contaminants in biosolids

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Prepared for Pacific Organic Residuals Conf.

UC Davis, CA

Sept 25-27, 2007

Introduction

- Macrolide antibiotics
  - Azithromycin -
  - Roxithromycin -
  - Clarithromycin -

Ranking among **all drug** prescriptions dispensed (US)

2005	2004	2003	2002
7th	8th	5th	6th

(widely prescribed Europe & Latin America)

206th

- Possible environmental sinks
  - Wastewater effluents
  - Biosolids
  - Sediments
  - Plants

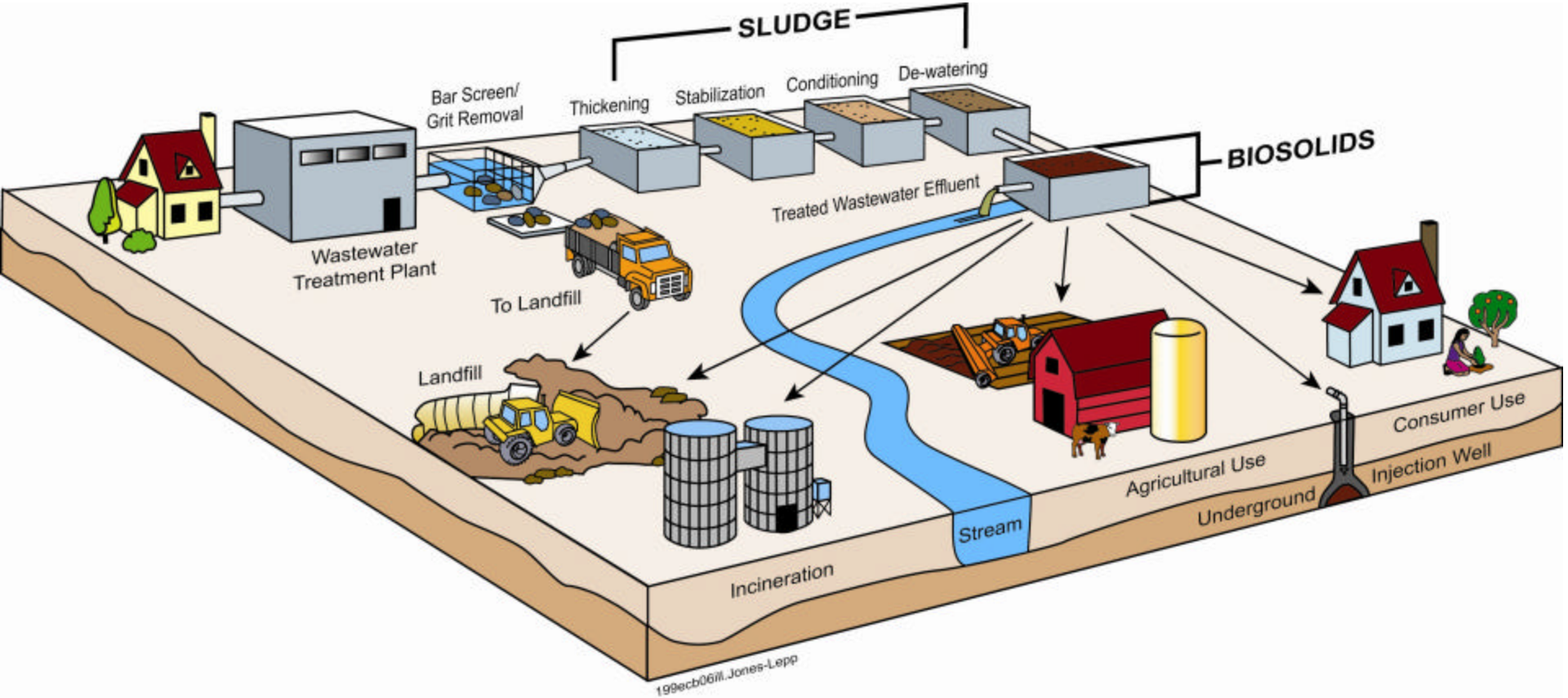
Possible environmental sinks

Biosolids

EPA's regulatory biosolids time-line

- **In 1993** the EPA promulgated the 40 CFR Part 503, *Standards for the Use or Disposal of Sewage Sludge*
- **In July 2002**, the NRC published a report entitled *"Biosolids Applied to Land: Advancing Standards and Practices"* in response to the EPA's request to the National Research Council (NRC) of the National Academy of Sciences (NAS) to independently review the technical basis of the chemical and microbial regulations applicable to sewage sludge applied to land
- **On 31 December 2003**, the Agency announced its final response strategy, also known as the Final Action Plan
  - EPA's final strategy in the *31 December 2003 Federal Register Notice* indicated that while emphasis was being placed on pathogens to address areas of uncertainty and public interest, selected new chemicals would also be addressed to help determine significant issues and identify information gaps that remain to be addressed
  - Some PPCPs are among those chemicals that EPA intends to study.

Production and distribution of biosolids



Agricultural application of biosolids

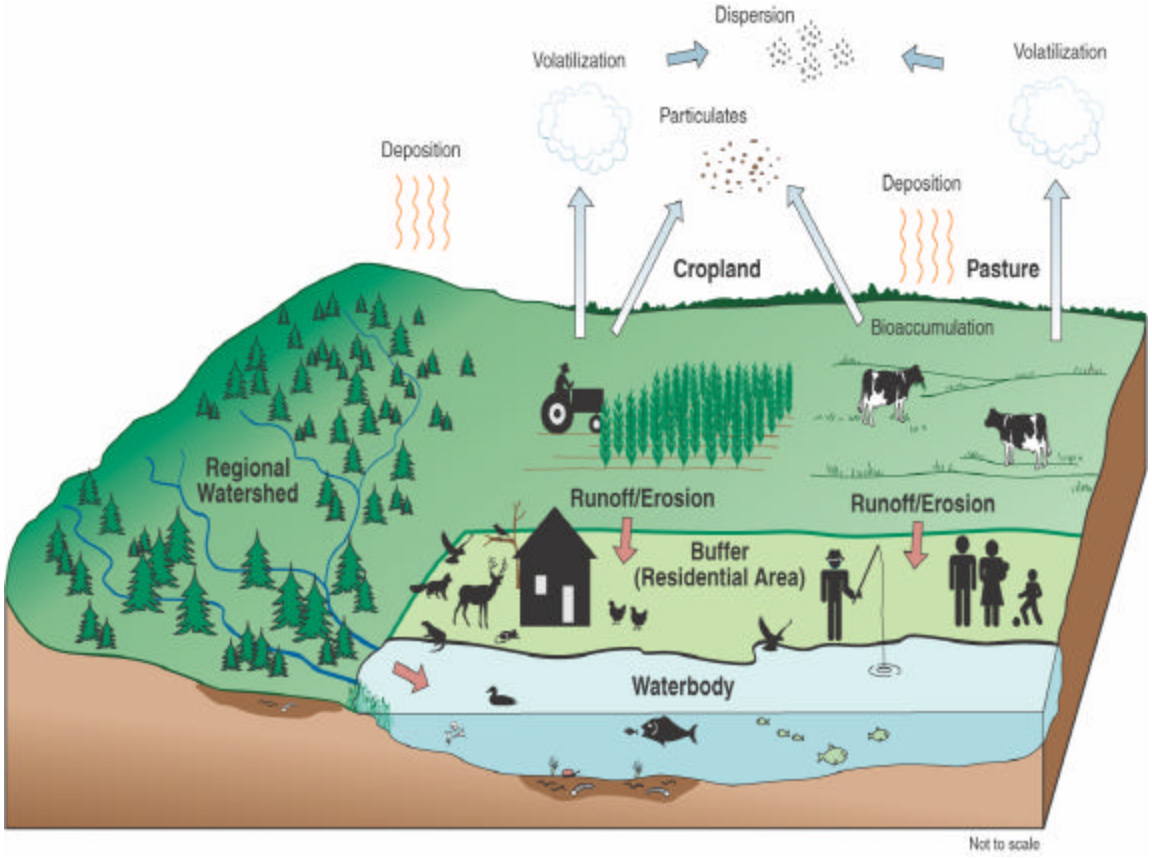


Figure courtesy of Rick Stevens, EPA, Office of Water

Why do we care about antibiotics??

- Possible adverse effects due to constant exposure of organisms to antibiotics
  - Increasing occurrence in the environment of antibiotic-resistant bacteria

- Miyabara, M. Imoto, S. Arai, J. Suzuki, S. Suzuki, **Distribution of antibiotic resistant Staphylococcus aureus in river water**. *Environ. Sci.,* 1995, **8**, 171.
- T. Schwartz, W. Kohnen, B. Jansen, U. Obst, **Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms** FEMS Microbiology Ecology 2003**, 43**, 325.
- Schwartz, T., Volkmann, H, Kirchen, S., Kohnen, W., Schon-Holz, K., Bernd J., Obst U., 2006. **Real-time PCR detecton of Pseudomonas aeruginosa in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates**. FEMS Microbiology Ecology 57, 158-167.

Chemical structures

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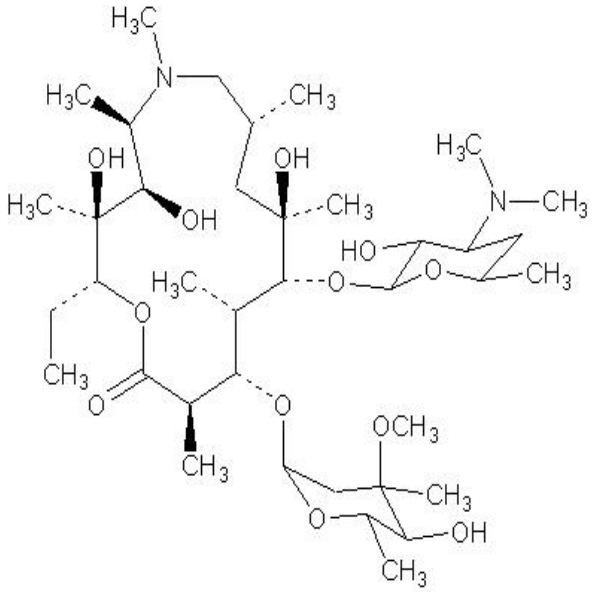
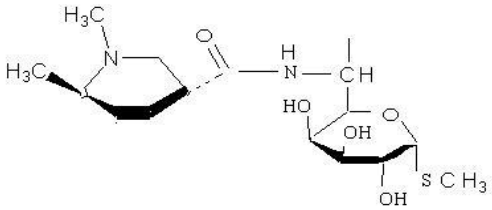
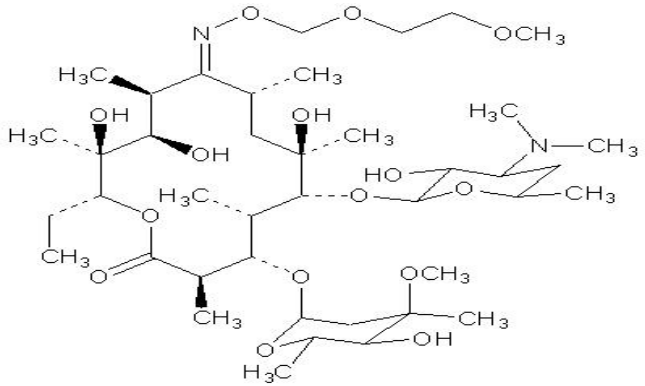
Azithromycin mw = 748.98 Da



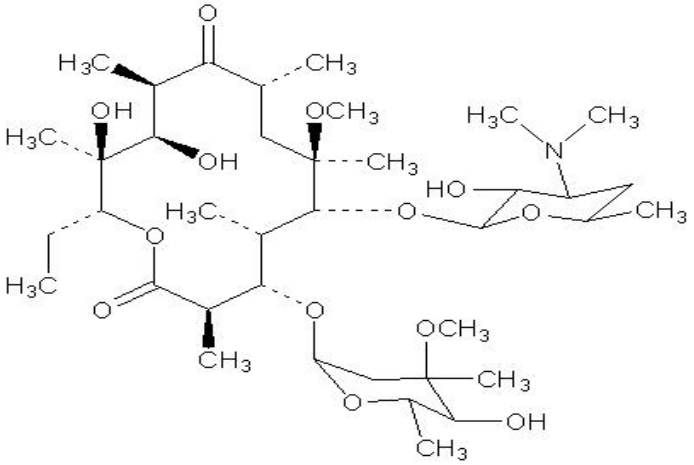


Clindamycin mw= 424.99 Da

Roxithromycin mw = 837.04 Da



Clarithromycin mw = 747.95 Da



Experimental

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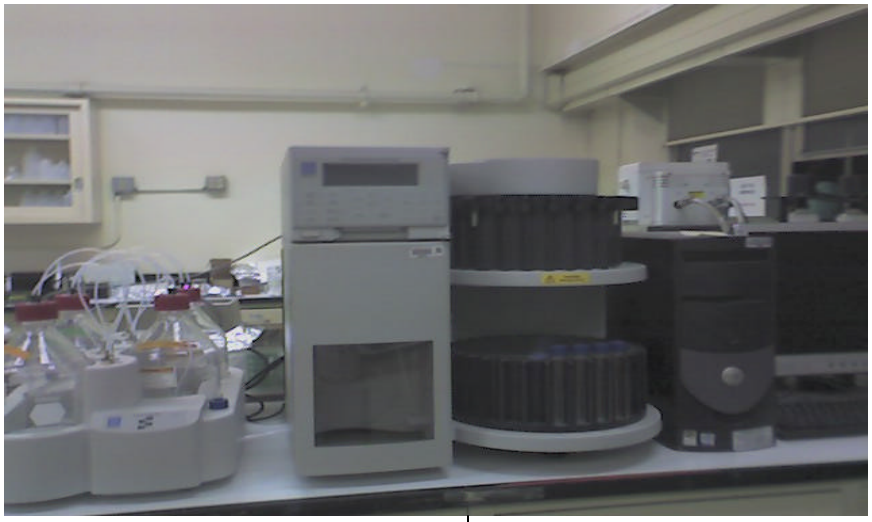
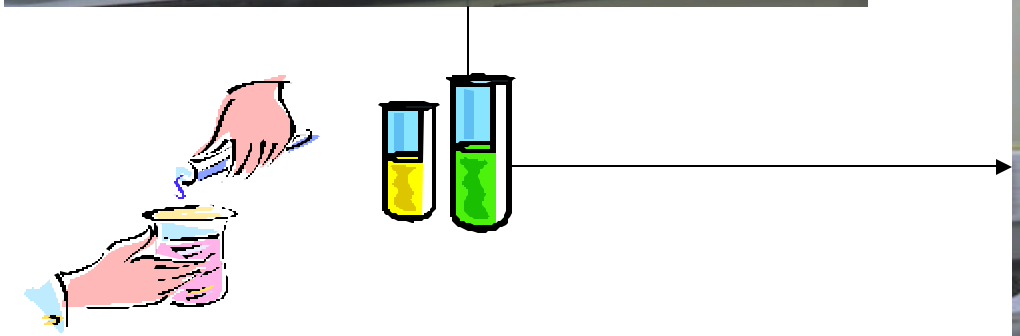
Pressurized liquid extraction (solids)

•Accelerated Solvent Extraction (ASE) system (Model ASE200, Dionex Corporation, Sunnyvale CA), 33-mL cell size

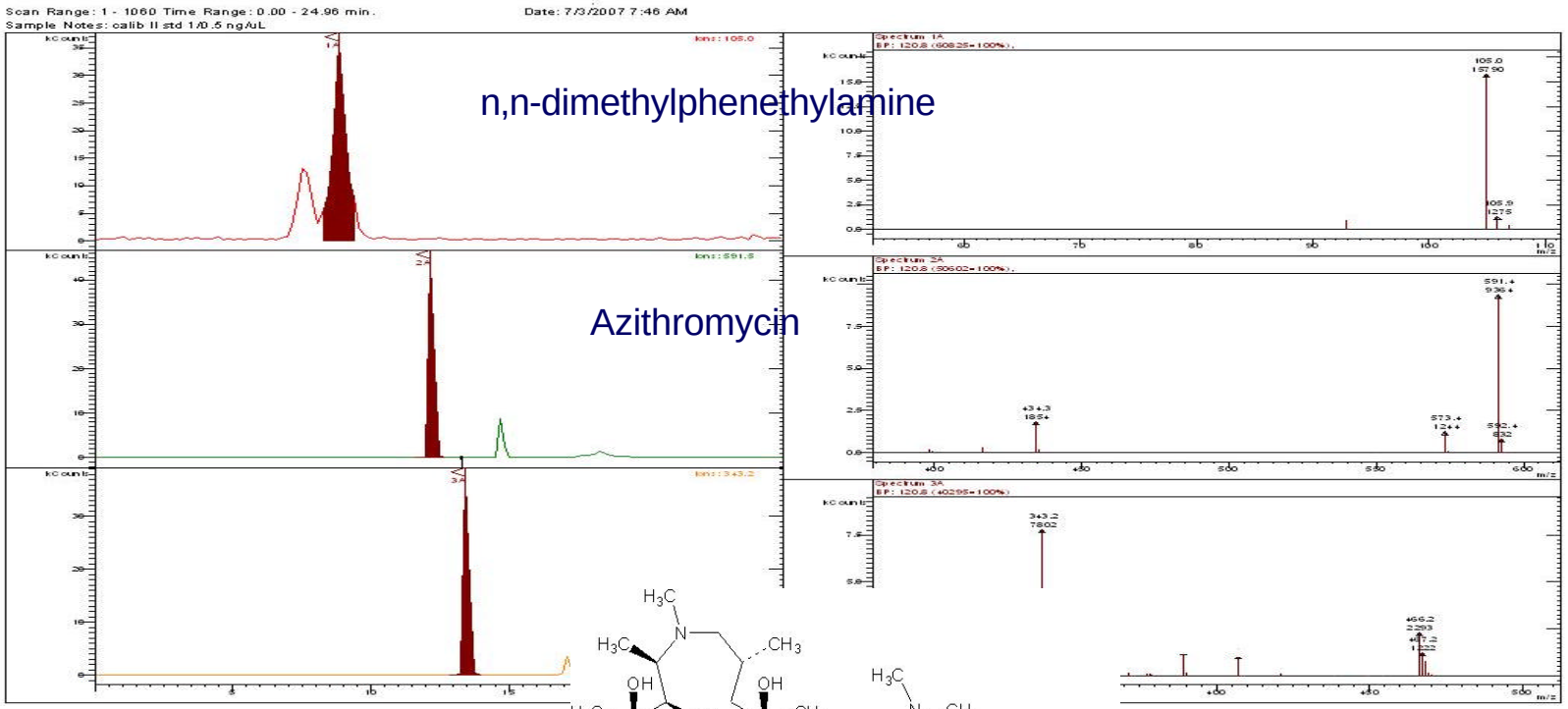




Hexane clean-up

Detection - Varian 500MS Electrospray ionization-ion trap mass spectrometry

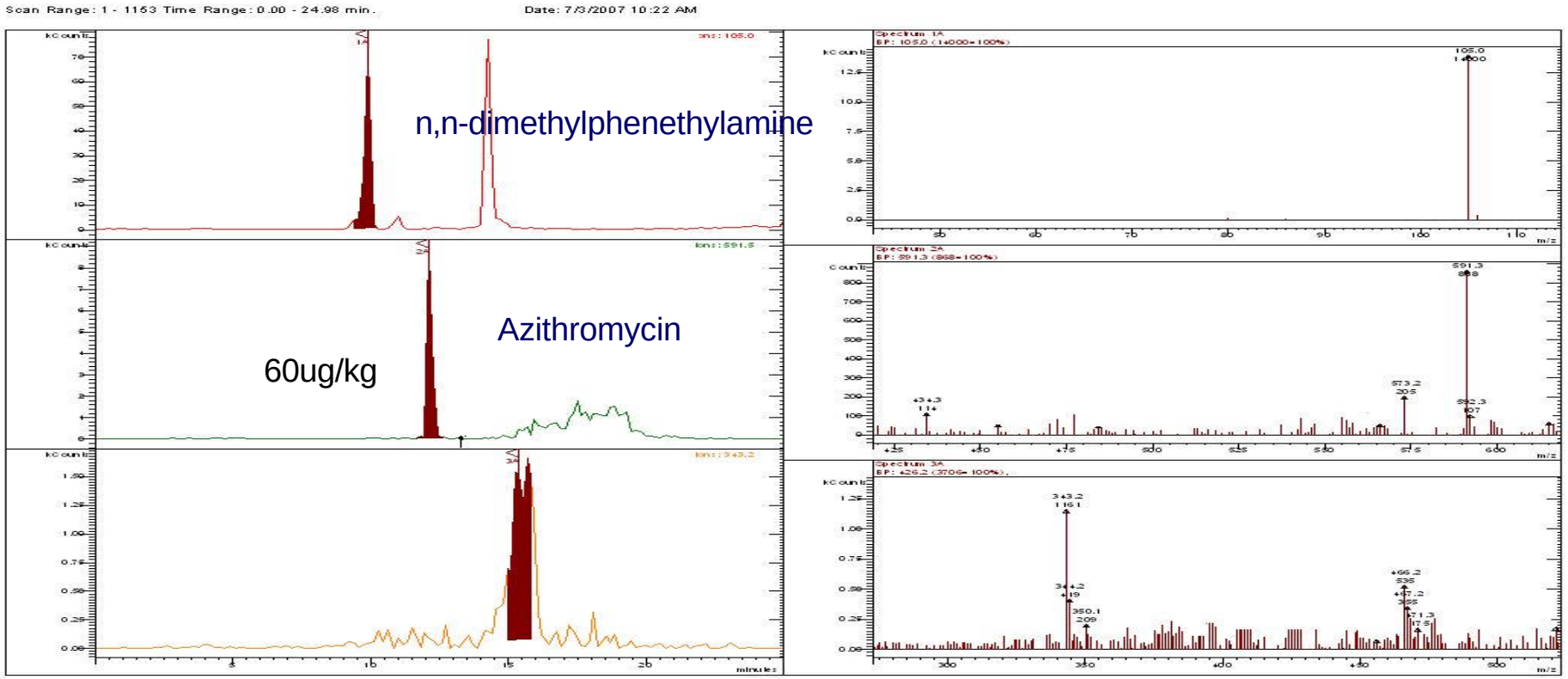
Example standard mass chromatogram and ms/ms spectra



Azithromycin mw = 748 Da

Example sample mass chromatogram and ms/ms spectra

Class B biosolid, small East Coast WWTP (3.3 mgd)



Biosolids results

ug/kg

DRAFT

Type	End-use	Urobilin	Azithro	Meth	MDMA	n,n-dmpea
Class B (can be considered Class A)	Landfill	0	8	0	0	0
dup		0	4	<LOD	0	1.5
Class A	Commercial product – land application	0	3	0	29	0
dup		0	2	0	25	0
Class B anaerobic digestion	incineration/land fill	47	53	0	0	22
dup		22	67	0	0	64
Class B anaerobic mesophilic digestion	Land applied	13	17	4	<LOD	0
dup		18	24	3	0	0

Azithro= azithromycin; Meth = methamphetamine; MDMA = Ecstasy;

n,n-dmpea = n,n – dimethylphenethylamine; LOD = limit-of-detection

Biosolids results cont.

ug/kg

DRAFT

Type	Urobilin	Azithro	Meth	MDMA	n,n-dmpea
Class B anaerobic digestion	34	9	<LOD	<LOD	0
dup	59	9	<LOD	<LOD	0
Class B anaerobic digestion	45	13	5	<LOD	0
dup	28	7	5	<LOD	0
Class B activated sludge	0	20	0	0	2
dup	0	18	0	0	2
Class B anaerobic digestion	3	4	0	0	3
dup	3	3	0	0	3

Other possible non-aqueous sinks
Sediments & Native Plants

Clark County WWTP outfall – August 2006	Azithromycin ng/g dry wt	Roxithromycin ng/g dry wt	Clarithromycin ng/g dry wt
Sediment 1	1 (28)*	detected < LOQ	77
Sediment 2	2 (64)*	1	97
Leafy material (tentative ID - *Nicotiana tabacum*; Cultivated Tobacco)	2	nd	nd
Root material	39	nd	nd

nd = not detected; *value in parenthesis reflects a “corrected” value base on % recoveries from each solid material

Conclusions

- Azithromycin was detected in all biosolids, ranging from 2 to 59 ug/kg.
- Recoveries are low due to interferences in the matrix (i.e., surfactants, fats)
  - Spiked matrix should be used to correct for low recoveries.
- Preliminary data suggests that there are reservoirs of the macrolides other than wastewater and biosolids, i.e., wetland plant/roots and sediments.
  - More wetland plant samples and sediments are needed to strengthen hypothesis.
- Correlation between prescribed use of macrolides and environmental findings.
- Illicit drugs were detected in 50% of biosolids.
- Finding illicit drugs [i.e., methamphetamine, MDMA (Ecstasy)] shows drug use other than just prescription/over-the-counter.

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On-going & future research

- Survey of sediment, soil and plant materials, where exposed to wastewater/biosolids, for macrolide antibiotics.
- Collaborative project for the analysis of biosolids for radionuclides.
- Analysis of wastewaters/source waters for water re-use project in lower Colorado River Basin
- Analysis of water, soils, crops from lower Colorado River Basin

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Acknowledgments

Thanks to Mr. Tom Moy, Senior Environmental Employee (SEE), for all of his hard work and dedication in the laboratory to make this project a success.

NOTICE

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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